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Genetic Effects of X-ray in Combination with Streptomycin on *Arabidopsis thaliana* I

SUJIT K. DHAR¹

Abstract. Soaking seeds of *Arabidopsis* in 0.005% streptomycin (SM) produced no second generation chlorophyll mutants. But when seeds were soaked in 0.005% SM for 20 hours before irradiation with X-ray, the percentage of chlorophyll mutants was lower than in plants obtained from seeds that were soaked in distilled water before exposure to X-ray. This protective action of SM was antagonized by 0.01 M $MnCl_2$. It is suggested that this change in the radiosensitivity of *Arabidopsis* by SM is caused by chelation of Mn^{++} with SM, thus causing a deficiency of the Mn^{++} ion, which is essential for the integrity of the chromosomes.

Lethal mutants, although frequently appearing in flowering plants, have been regarded with little interest; and with the exception of chlorophyll mutants no systematic study of their frequency or physiology has been made. The reason for this is that precise work on the organic nutrition of intact flowering plants is technically difficult.

In recent years, *Arabidopsis thaliana* has been increasingly used for the study of the induction of mutations in higher plants because it has a short life cycle, produces abundant seeds, and is self-pollinated.

Until 1927, all mutations, whether genic or chromosomal, were supposed to have been of spontaneous origin. In that year, H. J. Muller demonstrated that X-rays produce mutations in the sperm cells of *Drosophila*. Various factors have been known to influence radiosensitivity of organisms (Nilan, 1956, Konzak, 1957). Working with *Antirrhinum*, Sparrow et al (1961) found the larger the nuclear volume, the more sensitive the cell to X-ray. McKelvie (1962) found that Synkavit alone does not act as a mutagen, but when used with X-ray the percentage of mutation is much higher than with X-ray alone. Moutschen-Dahmen (1963) observed that the presence of copper, zinc or both during treatment with EMS (Ethyl Methane Sulfonate) increased the rate of chromosome breakage. Bhatia and Narayanan (1965) found a higher percentage of chlorophyll mutants when EMS was used along with copper or zinc. Alexander (1960) noted that incorporation of 5-bromodeoxyuracil into the DNA of cells renders the cells radio-sensitive. Hazama et al (1963) reported protective action of metallic ions such as Mn^{++} , Co^{++} , Ni^{++} , and Cu^{++} on radiation sensitivity of seeds. Bhatia and Narayanan (1965) found 7% chlorophyll mutants in the F_2 generation when EMS was buffered at pH5, but at pH9, they got 4% mutation. Alexander (1965) reported that

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addition of a reducing compound, such as cysteine, protects cells against radiation.

MATERIALS AND METHODS

Arabidopsis thaliana, family Cruciferae, was used in this investigation. The original seed supply was obtained from Oak Ridge National Laboratory, Tenn. Seeds were soaked in 0.005% streptomycin (SM) for 20 hours before irradiation. To test the effect of Mn^{++} , seeds were soaked in 0.01 M $MnCl_2$ for 10 hours before soaking the seed in SM for 10 hours. Another batch of seeds were soaked in SM for ten hours before soaking them in $MnCl_2$ for 10 hours. Yet another batch of seeds were soaked in 0.005% SM to which was added 0.01 M $MnCl_2$. Controls were soaked in distilled water for 20 hours. Irradiation given was 10Kr and 20Kr. Each different lot of seeds were irradiated at each of the two levels of X-ray intensity. Seeds were planted in steam-sterilized soil and kept in a controlled green-house environment under continuous illumination. To compute the percentage of first generation (X_1) survival, a count of all the seedlings was performed the fifth day after planting. For isolation of mutants in the second generation (X_2) bulk progeny, seeds were collected from individual X_1 rows. The X_2 plants were grown in a medium with chelated iron and other trace elements previously described (Dhar, 1969). This inorganic medium was solidified with 0.5% agar. The seeds were sterilized with 1:1 H_2O_2 and ethyl alcohol, then counted on a filter paper; and finally, with the help of a disposable pipette, the sterilizing solution was slowly dropped over the seeds so they got 10 minutes of sterilization time. The medium was disposed in petri dishes and autoclaved for 10 min. at 15 lbs pressure. The seeds were planted with a platinum loop, and the petri dishes were then kept at 0°C for 48 hours to insure uniform germination (Dhar, 1967).

RESULTS

Soaking seeds in SM before irradiation lowered the chlorophyll mutation rate (Table 1). Mn^{++} had an antagonistic effect on the protective action of SM (Table 1). However, the antagonistic effect of Mn^{++} was visible only when Mn^{++} was present during seed soaking in SM or when seeds were soaked in $MnCl_2$ solution after soaking in SM (Table 2). Soaking seeds in $MnCl_2$ prior to soaking them in SM did not antagonize the effect of SM (Table 2).

DISCUSSION

It may not be out of place to discuss the mechanics of SM action in plants since SM plus X-ray show some effects different

Table 1. Effect of streptomycin on chlorophyll mutation rate of X-ray.

| no streptomycin | | | streptomycin | | | streptomycin plus manganese | | |
|--------------------|----------------|-----------------------------------|--------------------|----------------|-----------------------------------|-----------------------------|----------------|-----------------------------------|
| total no. of seeds | no. of mutants | per cent of X ₂ plants | total no. of seeds | no. of mutants | per cent of X ₂ plants | total no. of seeds | no. of mutants | per cent of X ₂ plants |
| 0 Kr. 5000 | 0 | 0 | 5000 | 0 | 0 | 5000 | 0 | 0 |
| 10 Kr. 5000 | 204 | 4.1 | 5000 | 125 | 2.5 | 5000 | 200 | 4 |
| 20 Kr. 5000 | 205 | 5.0 | 5000 | 151 | 3.0 | 5000 | 253 | 5.0 |

Table 2. Effect of Mn⁺⁺ in combination with streptomycin on the chlorophyll mutation rate of X-ray.

| streptomycin and manganese together | | | streptomycin 10 hrs. then manganese 10 hrs. | | | manganese 10 hrs. then streptomycin 10 hrs. | | |
|-------------------------------------|----------------|-----------------------------------|---|----------------|-----------------------------------|---|----------------|-----------------------------------|
| total no. of seeds | no. of mutants | per cent of X ₂ plants | total no. of seeds | no. of mutants | per cent of X ₂ plants | total no. of seeds | no. of mutants | per cent of X ₂ plants |
| 0 Kr. 5000 | 0 | 0 | 5000 | 0 | 0 | 5000 | 0 | 0 |
| 10 Kr. 5000 | 200 | 4 | 5000 | 205 | 4.1 | 5000 | 121 | 2.4 |
| 20 Kr. 5000 | 253 | 5 | 5000 | 249 | 4.9 | 5000 | 150 | 5.0 |

from either X-ray or SM alone. SM has two general effects in plant tissues; it inhibits growth, and it bleaches green parts by interfering with the production and/or accumulation of chlorophyll in potentially green plants (Dhar, 1967). It was observed that bleaching is not an all-or-nothing affair and that the minimum concentration causing complete bleaching by soaking the seeds for 24 hours is 0.01%. No visible effect was observed on plants derived from soaking the seeds in 0.005% SM for 24 hours. In other words, SM, at the concentration used here, does not act as a "mutagenic" agent when used alone, but when used with radiation, it has some effects.

The mechanism of SM-bleaching in plants is not known. Rosen (1954) suggested that SM forms a chelating substance with manganese, rendering these ions unavailable to the plants. SM had a protective effect on the second generation chlorophyll mutation rate. However, when manganese was present during seed soaking in SM, the protective action of SM was reversed (Table 1, 2). It is suggested that this change in the radiosensitivity of *Arabidopsis* by SM is caused by chelate formation of Mn^{++} by SM, thus causing a deficiency of the ion in the seeds. Metallic ions like calcium and magnesium are essential for the integrity of the chromosomes (Steffensen and Bergeron, 1959). Von Rosen (1957) attributed mutagenic action of metal ions to enzymatic imbalance caused by deficiency or excess of metal ions.

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